

Anaphylaxis caused by cloxacillin: Diagnosis with seriated analysis by way of basophil activation test

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ABSTRACT

We report a case of anaphylaxis caused by cloxacillin in a 13-year-old patient. The basophil activation test, performed 25 days after the anaphylactic reaction, was positive to cloxacillin, amoxicillin, and penicillin G and negative to ibuprofen, tolerated by the patient. The analysis was performed 17 days after the reaction was not conclusive because 74% of the basophil population was activated in basal conditions. The abnormally high activation was similar to that found in an analysis before the reaction, exactly 4 days after finishing a well-tolerated treatment with amoxicillin. This first analysis was available because a patient's sample was taken from the emergency laboratory as a blind control for a study to assess the basophil activation test reliability in diagnosis of hypersensitivity to NSAIDs. The high number of activated basophils in basal conditions after treatment with amoxicillin and before the anaphylactic reaction to cloxacillin probably reflects the beginning of the sensitization. Until now, no cases of hypersensitivity to cloxacillin have been diagnosed by means of the basophil activation test.

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Allergic reactions to β -lactams are the most common cause of adverse drug reactions mediated by specific immunologic mechanisms.¹ Today, these reactions can be evaluated by prick and intradermal testing, with a sensitivity of 22–43% and a specificity of 97–99%. *In vitro* tests are available, such as the quantification of IgE-antibodies (FEIA, ImmunoCAP 250, Phadia, Sweden), with a sensitivity of 37.9% and a specificity of 86.7%.¹ Despite its limitations, the drug provocation test is still considered the “gold standard.”² To date, there is no reliable *in vitro* technique that confirms the diagnosis accurately. Therefore, the development of new *in vitro* diagnostic tests to avoid the risks of a provocation would be clinically relevant.

The basophil activation test is an *in vitro* diagnostic test that allows the quantitative determination of basophil degranulation, caused by the activation of the cell, in whole human blood after incubation with an allergen.³ After activation the tetraspan protein CD63 is expressed on the plasma membrane. The evaluation of basophil activation is performed by flow cytometry, which can analyze a large number of cells.^{4–6} The analyses of the activated basophils is performed on a double staining with monoclonal antibodies, anti-IgE,

to select the basophil population, and anti-CD63 to select the activated basophils.

The usefulness of this test in diagnosis of hypersensitivity to inhalants,^{7–9} foods,^{10,11} hymenoptera venom,¹² latex,¹⁰ and several drugs such as muscle relaxant¹³ and magnesium metamizol¹⁴ has been assessed in several studies. In allergy to β -lactam antibiotics, a sensitivity of 50% and a specificity of 93.3% have been achieved.^{15–18} To date, none of the cases published have used this method for studying the beginning of the sensitization.

CLINICAL CASE

We report the case of a 13-year-old male patient, atopic, sent from the Pediatric Department for presenting an anaphylactic reaction caused by cloxacillin. He was receiving this treatment for osteitis in the right arm. Ten minutes after the endovenous administration of 500 mg of cloxacillin he noticed generalized itching, dyspnoea, and dysphagia and presented a cough and a papular rash. These clinical symptoms subsided with inhaled adrenaline, antihistaminics, and corticosteroids. An hour earlier he had taken ibuprofen, which he tolerated later, and there was no food intake the 4 hours earlier. Hypersensitivity to latex was excluded.

Previous to his hospitalization he was diagnosed with acute pharyngitis and he followed treatment with amoxicillin for 8 days, noticing only dizziness while taking it. Four days after finishing the treatment fever persisted and he noticed pain in the right shoulder and biceps brachii, so he was taken to the emergency unit where symptomatic treatment with ibuprofen was rec-

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ommended. The day he attended the unit a blood sample was taken as a blind control (with another random samples) for a validation study of the basophil activation test in diagnosing hypersensitivity to non-steroidal anti-inflammatory drugs (unpublished data). He was selected as a blind control because he did not refer to any allergy to drugs, he was not following any treatment at that moment, and fever was not proved in the emergency unit.

METHODS AND RESULTS

The total IgE was higher than 2000 kU/L and the specific IgE (CAP Pharmacia, ImmunoCAP 250) was positive to penicillin G (2.49 kU/L), penicillin V (6.46 kU/L), ampicillin (0.47 kU/L), and cefaclor (0.89 kU/L) and negative to amoxicillin. The basophil activation test was performed with the commercial kit Basotest (Becton Dickinson, Pont de Claix, France).

A peripheral blood sample was drawn into heparinized tubes. One hundred microliters was necessary per test and was distributed in cytometry tubes, one for the negative control, one for the positive control, and one for each concentration of the tested drugs. Twenty microliters of stimulation buffer was added to each sample and incubated at 37°C in a water bath. One hundred microliters of washing solution (PBS solution) was added to one tube as the negative control. One hundred microliters of the chemotactic peptide fMLP (*N*-formyl-metionyl-leucyl-phenylalanine) working solution, which induces non-IgE-mediated basophil activation, was added to another sample as a positive control. The drugs tested were penicillin G (Penibiot; Normon Laboratories, Madrid, Spain), amoxicillin (Clamoxyl; SmithKline Beecham Laboratories, Madrid, Spain), cloxacillin (SmithKline Beecham Laboratories, Madrid, Spain), ibuprofen (Espidifen; Zambon Laboratories, Barcelona, Spain), and lysine acetylsalicylate (Inyesprin; Andromaco Laboratories, Madrid, Spain), all at concentrations of 1 $\mu\text{g}/\mu\text{L}$ and 100 ng/mL. One hundred microliters of these dilutions were added to both samples. The tubes were incubated for 20 minutes at 37°C in a water bath, time in which the degranulation took place if the basophil became activated. This process was stopped, incubating the samples on ice for 5 minutes. Twenty microliters of staining reagent, containing PE (Phicoerythrin)-conjugated anti-IgE and FITC (Fluorescein)-conjugated anti-gp53 (also known as anti-CD63) were added to each sample. They were later incubated for 20 minutes in an ice bath preserved from light exposure. Next, the whole blood samples were lysed with 2 mL of prewarmed lysing solution (diluted 1:10) for 10 minutes at room temperature. They were centrifuged at $250 \times g$ for 5 minutes. The supernatant was aspirated and the samples were washed with 3 mL of washing solution and centrifuged again. After decanting the supernatant, the samples were resuspended in 400 μL of

PBS solution and analyzed within an hour by means of cytofluorometry. Acquisition was performed on a minimum of 800 cells/sample. The analysis of surface markers was performed on a flow cytometer (EPICS XL-MCL; Beckman Coulter, Miami, FL) using the Lysis II Software. The analysis gate was defined around cells showing high-density anti-IgE label, identified as basophils. We assessed that the size and granulation of these cells was as expected (just between lymphocytes and monocytes) on a histogram defined by forward scatter and side scatter. The expression of CD63 was analyzed on this gated cell population. The results were given according to the following formula: percentage of double-positive IgE⁺ and CD63⁺ cells after drug stimulation/percentage of double-positive IgE⁺ and CD63⁺ cells in the negative control. This coefficient is called the *Basophil Activation Index* and when it is equal or higher than two the result of the assay is considered positive.

Four days after finishing treatment with amoxicillin, a basophil activation test with lysine acetylsalicylate was performed on this patient as a blind control (with another random sample) to study this method's usefulness in diagnosis of hypersensitivity to nonsteroidal anti-inflammatory drugs (Rodríguez Trabado A, Fernández Pereira L, Porcel Carreño SL, et al. Use of the basophil activation test to diagnose hypersensitivity to non-steroidal anti-inflammatory drugs in vitro. Submitted for publication). The result was considered as not valuable because an activation of the 40.6% of the basophil population was seen in basal conditions (Fig. 1) Seventeen days after the anaphylactic reaction to cloxacillin a new analysis was performed with cloxacillin and ibuprofen. This result was not valid to diagnose the culprit drug of this anaphylactic reaction because a similar activation in the basophil population of 74.9% of the cells was seen, although both results made us think that, in fact, we were seeing the sensitization process (Fig. 1).

The study performed 25 days after the adverse reaction was diagnostic of hypersensitivity to penicillin G, amoxicillin, and cloxacillin; and a significant activation of the basophils was not shown after stimulation with ibuprofen, tolerated after the reaction (Table 1). In two healthy controls the test was negative to cloxacillin. To reject other causes of high basophil activation as confusion factor, two patients with infectious disease, gastroenteritis and acute tonsillitis, and fever were studied. They were not taking any treatment before taking the blood sample. None of them showed high basal basophil activation and the response to the positive control, fMLP stimulus, was appropriated. Then, fever was rejected as confusion factor causing nonspecific basophil activation.

Thus, test specificity in patients with high levels of total IgE was studied also. Two brothers with hyper-IgE syndrome had positive results to the allergens that caused clinical manifestations, grass pollen, and *Alter-*

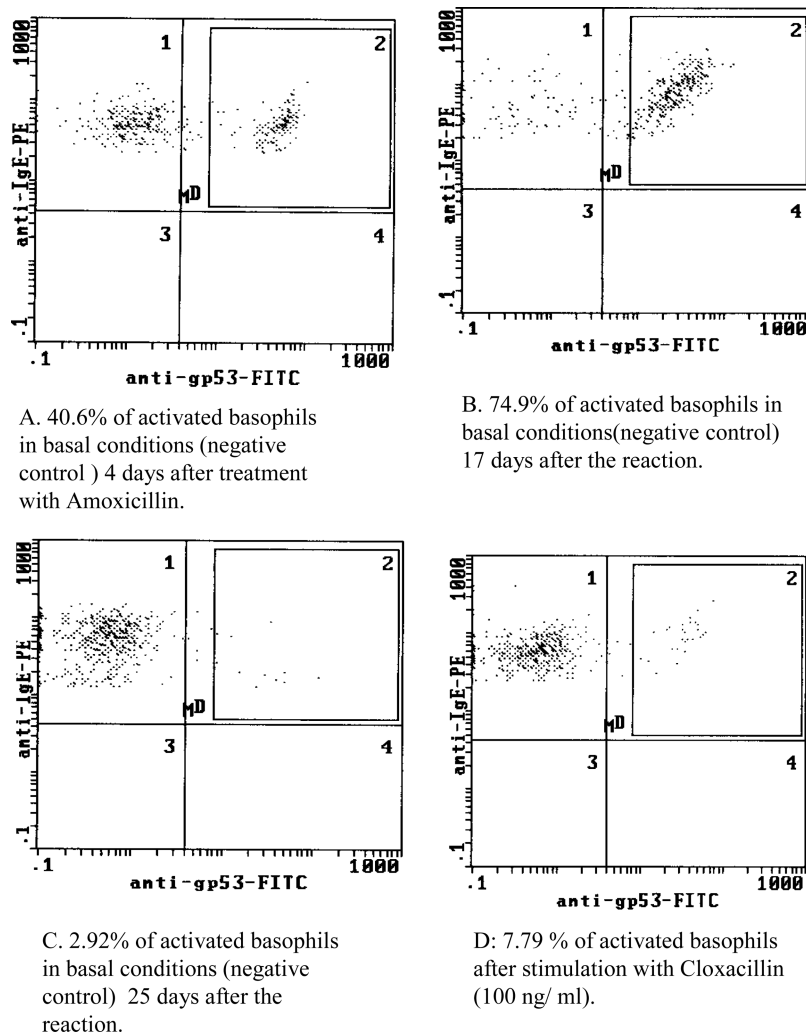


Figure 1. Seriated analysis by way of the basophil activation test.

Table 1 Seriated analysis by way of the basophil activation test

Date	Negative Control	Positive Control	Drug	Activated Basophils (%)
25 days after the anaphylaxis	2.9%	34%	Cloxacillin	7.79
			Penicillin G	9.54
			Amoxicillin	28.5
			Ibuprofen	5.02
17 days after the anaphylaxis	74.9%	82.3%	Penicillin G	62.9
			Ibuprofen	67.5
4 days after treatment with Amoxicillin	40.8%	75%	Lysine acetylsalicylate	17.8

naria alternata and negative results to drugs tolerated by them.

DISCUSSION

We report a case of anaphylaxis due to cloxacillin, which was caused by an allergic mechanism assessed by the basophil activation test. With this test, adverse immediate reactions, IgE or non-IgE mediated, in

which the basophils play an important role, can be assessed. The basophil activation test increases the diagnostic possibilities because many drugs can be *in vitro* tested in comparison with the available tests we have today, such as the determination of specific IgE to many β -lactam antibiotics.

In normal conditions, no more than 5% of the basophils are activated in the absence of antigenic stim-

ulus,¹⁹ as was seen in the analysis performed 25 days after the anaphylaxis. From a physiopathological point of view, it is extremely interesting to have observed the activation of a high number of the basophil population in basal conditions 17 days after the anaphylactic reaction, similar to the activation shown 4 days after finishing the treatment with amoxicillin, the drug that is supposed to be the primary sensitizer. This activation persisted 2 weeks after the reaction and a normal percentage of activated basophils was shown 25 days after the reaction. This high degree of activation could be related to the behavior of the basophil population while an allergic reaction is taking place and seems to persist for many days once the reaction has occurred.

In our case, we could directly observe these changes before the anaphylactic reaction as a finding when we analyzed the blood sample as a blind control for the validation study of the basophil activation test. The normal values of basal basophil activation seen in the two patients with fever and infectious disease studied, leads us to think that these two conditions had no influence in the abnormally high percentage of the basophil population activated in basal conditions in our patient's case. Cloxacillin did not induce a positive response in the two healthy control samples; therefore, unspecific basophil activation due to this drug was not expected. Indeed, the test is strongly specific in patients with high levels of total IgE in blood, without interference in the basophil population isolation.

The main aim of this test has not been the detection of sensitization to previously tolerated drugs. Nevertheless, it could be used to study the basophil behavior after a drug provocation test to predict future reactions due to immunologic memory reactivation, a possibility noticed with many drugs such as β -lactam antibiotics.¹

In our case, the basophil activation test assessed the diagnosis of the anaphylactic reaction caused by cloxacillin. The high number of activated basophils in basal conditions after treatment with amoxicillin and before the anaphylactic reaction to cloxacillin probably reflects the beginning of the sensitization; this opens a way to prevent drug reactions, an exciting new field for drug allergy diagnosis. Until now, no cases of hypersensitivity to cloxacillin have been diagnosed by the basophil activation test.

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